

# THE PATHOGENIC RICKETTSIAE WITH PARTICULAR REFERENCE TO THEIR NATURE, BIOLOGIC PROPERTIES, AND CLASSIFICATION

HENRY PINKERTON

*Department of Pathology, Saint Louis University School of Medicine, St. Louis, Missouri*

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The growth of our knowledge of the pathogenic rickettsiae in recent years forms one of the most fascinating chapters in the field of microbiology. Intensive study of these organisms has paved the way for the control of the important human diseases which they produce. Of still greater importance, perhaps, is the light which these studies have thrown on problems of intracellular parasitism in general.

Historically it is of interest that, following early unsuccessful attempts to solve the etiology of typhus and Rocky Mountain spotted fever by simple bacteriological methods, these infections were thought to be caused by viruses. Subsequently, minute intracellular organisms, morphologically similar to bacteria, were associated with these diseases. These organisms were first found in the tissues of the arthropod vectors. They were called rickettsiae, for reasons which will be brought out below, and were considered to belong to a new group of pathogenic agents, distinct from bacteria and viruses. Recent developments indicate that the rickettsiae have many features in common with certain bacteria and with some of the viruses and suggest that the attempt to separate these three groups of pathogens sharply from one another may be unwise. In order that the rickettsiae may be considered in proper perspective, a brief discussion of their inter-relationship with bacteria and viruses will be given.

## BIOLOGIC RELATIONSHIP TO OTHER PATHOGENS

Among the pathogenic organisms at present classed as bacteria, there is great variation in the ability to carry out independent metabolic activities (1). Certain organisms, like the colon bacillus, live and multiply freely in media containing simple carbon compounds, ammonia, and inorganic salts. These bacteria possess enzyme systems of a complex nature, by virtue of which they are able to carry out the chemical transformations necessary for their free life and

reproduction. Other bacteria, like the influenza bacillus, have a less complete enzymatic equipment, and therefore require more complex media containing organic substances which are found only in animal tissues or in extracts of animal tissues.

Organisms of the group last mentioned are, therefore, somewhat dependent for their food supply on living cells of the animal which they parasitize. Like those of the first group, however, they grow in the intercellular fluids of the infected animal and are practically never seen within cells, except insofar as they may be engulfed by the defensive phagocytic cells of the body. The cultivation of these organisms on bacteriological media, though somewhat more difficult than in the case of the colon bacillus, is accomplished with relative ease, because the compounds on which they live can be obtained in a fairly stable form by extraction from living tissues.

A still greater degree of dependence on living animal tissues is shown by organisms like *Pasteurella tularensis* and *Bartonella bacilliformis*. These organisms, in infected tissues, multiply extra-cellularly under certain conditions, but under other conditions show a decided preference for the interior of cells, multiplying freely in the cytoplasm. Their growth in bacteriological media is achieved only by the careful reproduction of certain necessary chemical and physical conditions. The beneficial effect of cystine in the cultivation of *P. tularensis*, for example, is of great interest because of the fact that this substance is of importance in intracellular oxidation-reduction processes. It is probable that in adding cystine to our artificial medium we are supplying a metabolic activator for the action of which the organism, in nature, depends on living cells of the animal which it infects. Similarly, Geiman (2) has recently reported that the addition of ascorbic acid and glutathione to culture media facilitates the growth of *Bartonella bacilliformis*.

The next stage in the biological adaptation to growth in living cells is represented by the obligate intracellular parasites. Conditions suitable for the multiplication of these pathogens are found only within the cytoplasm or nucleoplasm of living or surviving cells. This group includes the typical pathogenic rickettsiae and probably all of the viruses, although direct proof that certain invisible viruses multiply only within cells has perhaps not been furnished. In tissues infected with pathogenic rickettsiae, we find colony-like masses of organisms in the cytoplasm or nucleus of cells. Similar intracellular colony-like masses of elementary bodies are associated with the presence in tissues of certain of the larger viruses, such as psittacosis (32) (intracytoplasmic) and herpes (89) (intranuclear). In tissues infected with the smaller viruses, we often find spherical intracellular bodies (the so-called inclusion bodies) which appear homogeneous and which may or may not be agglomerated masses of elementary bodies, with or without the addition of other elements derived from the host cells. Limitations of microscopy have thus far made it impossible to determine the exact nature of many of these inclusions, but it seems possible that some of them may not differ essentially from colonies of rickettsiae or elementary bodies. The nature of the inclusion bodies associated with the

smallest viruses, which approach molecular size, remains a matter of speculation (3).

Present evidence indicates that the rickettsiae, although they are as strictly dependent on intracellular conditions as the smaller viruses, have a somewhat more complex enzyme system than the latter, and are therefore able to maintain a certain amount of independent metabolic activity within their host cells. Thus we may consider the rickettsiae, both from the point of view of size and from the point of view of independent metabolic activity (enzymatic complexity) as occupying a position intermediate between certain cytotropic bacteria and the viruses.

#### TAXONOMIC CONSIDERATIONS

*Historical.* In dealing with a new group of infective agents, it is inevitable that early attempts at nomenclature and classification, based upon incomplete knowledge of their biological properties, should lead to considerable confusion. Even at the present time, criteria suitable for a satisfactory classification are not available, and any attempt to classify the rickettsiae must be considered as having only a tentative and pragmatic value. In order to understand the taxonomic difficulties which obtain at present, a brief discussion of nomenclature from the historical aspect seems desirable.

The term "Rickettsia" was applied in 1916 by da Rocha-Lima (4) to certain minute microorganisms found in the intestinal tract of lice. The name honors the memory of Howard Taylor Ricketts, who with Wilder first described in 1910 organisms of this type in lice fed on typhus patients (5, 6). da Rocha-Lima showed, by studying sections of lice, that the organism acquired by feeding on typhus patients was an intracellular parasite, distending the epithelial cells of the louse's stomach. He believed this organism to be the etiological agent of typhus, and subsequent work, notably that of Wolbach, Todd, and Palfrey (7) published in 1922, has established the truth of this concept. To this organism, da Rocha-Lima gave the name *Rickettsia prowazeki*, the specific name being in honor of von Prowazek, who, like Ricketts, died of typhus acquired in the course of his pioneer investigations.

Organisms morphologically similar to *R. prowazeki* but extracellular in habitat were found in lice in association with trench fever by Töpfer (8, 9) in 1916, and in presumably normal lice by Munk and da Rocha-Lima (10) in 1917. The latter workers gave to these organisms the name *Rickettsia pediculi*, although they fully realized that they differed from *R. prowazeki* in that they multiplied extracellularly in the digestive tract of the louse. The probable relationship of such extracellular organisms to trench fever will be discussed below.

*Non-pathogenic rickettsiae.* During the next decade approximately forty-two microorganisms, some of intracellular and some of extracellular habitat, were described in the tissues of thirty-seven species of arthropods. Information concerning these organisms was in most cases confined to the mere fact that they could be seen in certain locations in the tissues of certain arthropods, and the possibility that the same organism might be present in two or more arthropods

was not excluded. It was natural that the general term rickettsia should be applied to these organisms, since for the most part they resembled *R. prowazeki* rather closely. The generic name *Rickettsia*, together with a specific name, was given to a number of them. In many cases, this was unfortunate. *Rickettsia melophagi* (11), for example, an extracellular organism found in the intestine of the sheep ked, and rather easily cultivated on ordinary bacteriological media, has little in common with the more characteristic rickettsiae, which are obligate intracellular parasites.

This large group of organisms in insect tissues is often referred to as the "non-pathogenic rickettsiae," a term indicating their lack, so far as our knowledge goes, of pathogenicity for mammals. For the most part, they are also non-pathogenic for their insect hosts, and in many instances are transmitted hereditarily from one generation to another by way of infected ova, apparently living in complete harmony with their host cells in the insect tissues.

Closely related to the rickettsiae, and at times not sharply separable from them, are the so-called symbionts, some of which appear to exert a useful or even essential function in the metabolism of insect tissues. For a general discussion of the microorganisms and other structures found in insect tissue, and of their relation to one another, the reader is referred to a recent paper by Steinhaus (12).

Presumably the rickettsia of typhus fever (*R. prowazeki*), and other pathogenic rickettsiae which will be discussed below, belong in the same general group as many of the non-pathogenic rickettsiae described above, and their pathogenicity for mammals is purely accidental or even acquired. Antigenic relationship between pathogenic and nonpathogenic rickettsiae has not, however, been demonstrated (13).

Most of the non-pathogenic rickettsiae have been identified and named entirely on the basis of their morphology and distribution in insect tissues. The limitations of this method of study are obvious. Organisms which look alike may be physiologically very different, and, since pleomorphism may occur in the rickettsiae, morphologically dissimilar organisms may actually be identical. Although many careful and valuable studies of a purely morphological nature have been made, and in some instances the assignment of generic and specific names may have been justified, the accurate classification of the non-pathogenic rickettsiae must in general await the development of methods for isolating them and for determining their biological properties.

*Pathogenic rickettsiae.*—The rickettsiae that are pathogenic for mammals may be isolated and identified much more readily than the non-pathogenic rickettsiae. When an organism invades mammalian tissues and produces constantly a characteristic infection, we may be sure that we are dealing with a definite entity. The tissues of ticks may contain non-pathogenic rickettsiae as well as *Derma-centroxenus rickettsi*, the cause of spotted fever, and the two types of organisms may, except for the invasion of nuclei by the latter, be morphologically indistinguishable (13). By feeding such ticks on guinea pigs, however, or by injection of the tick viscera into guinea pigs, we can readily separate the pathogenic organism from the non-pathogenic organisms, since only the pathogenic one

will invade the mammalian tissues. Immunological tests on guinea pigs recovering from rickettsial infection serve to identify the pathogenic rickettsiae with great precision. Attempts to define and classify the pathogenic rickettsiae have for these reasons been relatively successful.

Using as a basis the criteria suggested by Cowdry (14) in 1923, by Wolbach (15) in 1924, and by Cowdry (16) in 1926, the rickettsiae may be characterized as follows: *small, often pleomorphic, gram-negative, bacterium-like organisms, living and multiplying in arthropod tissues, behaving as obligate intracellular parasites, and staining lightly with aniline dyes. With few exceptions, criteria adequate for classification on the basis of biological properties are available only for those members of the group which are pathogenic for mammals.* At present, only four pathogenic organisms can be definitely placed in the group if we define it thus rigidly: *Rickettsia prowazeki*, the cause of louse- and flea-borne diseases of the typhus group; *Demacentrozetes rickettsi*, the cause of tick-borne diseases of the spotted fever group; *Rickettsia tsutsugamuchi* (synonym, *R. orientalis*), the cause of mite-borne diseases of the tsutsugamuchi group; and *Rickettsia ruminantium*, the cause of tick-borne "heartwater" in sheep, goats, and cattle.

Wolbach (17) in 1919 believing that the etiological agent of Rocky Mountain spotted fever showed more than species differences from *Rickettsia prowazeki*, the cause of typhus, gave the former organism a different generic name (*Demacentrozetes*). Subsequent work has substantiated the rather wide differences between the two organisms, and it is to be regretted that organisms more recently added to the group of rickettsiae have not likewise been placed in different genera since, almost without exception, they have shown differences of sufficient magnitude to make such assignment appear desirable.

The author (18) has suggested that the pathogenic rickettsiae, as a tentative working basis, be considered as members of a bacterial family, the RICKETTSIACEAE. (The reasons for not separating them from bacteria will be brought out below.) This plan allows for the creation of new genera, if this seems advisable, for newly discovered organisms which possess the necessary characteristics for inclusion in the group but which show differences from the type genus and species, *Rickettsia prowazeki*, of such importance that they are regarded as of more than specific value. The term rickettsiae, spelled with a small letter, has become firmly established in the literature and may be used loosely as a synonym for the RICKETTSIACEAE, much as we use the term actinomycetes for various species of ACTINOMYCETACEAE.

Although the family RICKETTSIACEAE was created for pathogenic rickettsiae, non-pathogenic organisms, when sufficiently well studied to show that they probably are distinct entities and possess the required characteristics, may tentatively be included. Hertig (19), for example, has described an organism of constant occurrence in the gonads of the mosquito, *Culex pipiens*. To this organism he gave the name *Wolbachia pipientis*, including it with the RICKETTSIACEAE. The non-pathogenic rickettsiae will not be considered in detail in this paper. For a detailed discussion of them, reference may be made to papers by Wolbach (15), Hertig and Wolbach (20), and Cowdry (16).

The etiological agent of Q-fever, named *Rickettsia burneti* and *R. diaporica*

by Australian and American workers, respectively, although a facultative rather than an obligate intracellular parasite, appears otherwise very closely related to the more characteristic rickettsiae listed above. As a matter of convenience, and with the realization that no entirely satisfactory definition of the rickettsiae can be given at present, this organism will be included with the rickettsiae for the purposes of this discussion. For the same reason and in spite of the fact it is apparently an extracellular organism, the probable etiologic agent of trench fever, *Rickettsia wolhynica* (*pediculi*, *quintana*), will also be discussed.

*Rickettsia canis*, *R. bovis*, and *R. ovina*, will also be reviewed briefly, for the sake of completeness, since it seems possible that these organisms may eventually be shown to possess the necessary characteristics for inclusion with the rickettsiae.

*Rickettsia-like pathogens.* A number of pathogenic agents have been described which resemble the rickettsiae in many ways, but which for one reason or another cannot at present be included with them. The biological properties of these organisms will be discussed briefly at this point, since a study of their relation to each other, to the typical pathogenic rickettsiae, to bacteria, and to viruses brings out facts which harmonize well with modern theories concerning the interrelationship of various groups of pathogens.

*Bartonella bacilliformis* is a facultative, rather than an obligate intracellular parasite, non-filterable, and morphologically and tinctorially greatly resembling the rickettsiae (21). It inhabits the tissues of and is probably transmitted by the bite of sand flies (22, 23), but the mechanism of transmission and the morphological picture of infection in the sand fly have not yet been cleared up (24). This organism has been cultivated on special cell-free media (25), but its close relative, *B. muris*, has resisted such cultivation. The similarity of these organisms to the rickettsiae has been pointed out by various workers (26). Their ability to produce severe anemia, by parasitizing the erythrocytes of their mammalian hosts, together with the lack of information concerning their behavior in the sand fly would appear to justify their exclusion for the present from the rickettsiae. It is of interest to note that *B. bacilliformis*, in plasma tissue cultures, forms extracellular colonies in the plasma clot as well as intracellular colonies (21). This indicates that its growth requirements are simpler than those of the typical rickettsiae (see below).

*Pasteurella tularensis* is a facultative intracellular parasite, non-filterable, and transmitted biologically<sup>1</sup> by ticks. This organism is so perfectly adapted to life in tick tissues that, like the spotted fever rickettsia, it is transmitted hereditarily from one generation to another by infection of the ova. It multiplies in and distends the epithelial cells lining the gut of the tick, producing a picture similar to that of *R. prowazeki* in the louse (26a). In contrast to *R. prowazeki*, however, it also grows freely in the coelomic fluid. The author has found (21) that in tissue cultures it grows both in the cytoplasm of cells and free in the surrounding plasma clot behaving much like *Bartonella bacilliformis*. In guinea

<sup>1</sup> That is, by actually multiplying in tissues, as opposed to purely mechanical transmission.

pigs it behaves rather conspicuously as a facultative intracellular parasite, massively infecting both the Kupffer cells and the liver cord cells (27). The organism has been cultured on special cell-free media (notably those containing cystine) and is of course classed with the bacteria, on the basis of its biochemical reactions. Its similarity to the rickettsiae, although obvious from the above discussion, has not been stressed, and it would serve no useful purpose at present to include it with these organisms.

It has been suggested that the elementary bodies of psittacosis (28) and those of trachoma (29) may be rickettsial in nature. Similar bodies are associated with lymphogranuloma venereum (30), and in all three conditions these structures are confined to the cytoplasm of cells. In the case of trachoma, insect transmission has been suggested (31). Unless insect transmission of these infections is conclusively demonstrated, however, these structures should not be considered as rickettsiae. Their complex morphology, which, in the case of psittacosis has been interpreted as evidence of a life cycle (32), suggests that they may differ considerably from the rickettsiae. Since psittacosis is at present classed as a virus disease, it is customary to refer to the intracellular structures associated with infection as elementary bodies, rather than organisms. The constant association of these discrete bodies with infectivity strongly suggests that they are the etiological agents of the infection, and it is probable that they may prove to be organisms in the same sense that rickettsiae are. If biological transmission by insects should be demonstrated, their inclusion with the rickettsiae would have to be seriously considered.

An intracellular organism associated with conjunctivitis in sheep and goats has been named *Rickettsia conjunctivae* by Coles (33), and a similar organism *R. conjunctivae bovis* was described by the same author in cattle (34). Another similar organism has been described, but not named, by Johnson (35) as the probable cause of a type of conjunctivitis in sheep. It seems unwise to class such organisms as these with the rickettsiae, since the most important single criterion for such assignment—development in insect tissues—has not been established, and it is clear that infection is commonly transmitted by direct contact.

Mochkovski (36) has made the interesting suggestion that the Foa-Kurloff bodies may be rickettsiae. Since he has furnished no proof that these structures are microorganisms, or that they inhabit insect tissues, this suggestion cannot at present be taken seriously.

The rickettsia-like organism described by Sellards and Siler (37) in association with dengue is probably non-pathogenic in nature.

*Interrelationships.* For many years the rickettsiae have been considered to represent a new group of microorganisms distinct from bacteria, a point of view which has proven useful and stimulating. From a consideration of the above discussion, the difficulties of drawing sharp lines of demarcation between bacteria, rickettsiae, and viruses become evident. Among rickettsia-like pathogens we have mentioned one (*Pasteurella tularensis*) which is accepted as a bacterium and variously grouped with *Pasteurella pestis* and *Brucella abortus*, and one

(psittacosis "virus") which is commonly classed as a filterable virus. The typical rickettsiae resemble bacteria in their visibility, morphology, and apparent non-filterability, but are close to the filterable viruses in their specificity for certain cell types and in their growth requirements. The modern tendency to regard viruses as organisms which have lost their enzyme systems by a process of evolution tempts one to cast the rickettsiae in the rôle of a missing link.

The inclusion of obligate intracellular parasites with the bacteria is not without precedent, however (for example, the lepra bacillus), and a more satisfactory concept would appear to be that of an unbroken series, with free-living bacteria at one end, the smallest viruses at the other end, and a large number of organisms and elementary bodies intermediate at many points between these two groups. On the whole, therefore, it seems most logical to regard the rickettsiae as bacteria which have become adapted to intracellular life in arthropod tissues. The alternative theory, that bacteria are viruses, originally imprisoned in cells, which have acquired the ability to lead an independent existence, seems less attractive.

In conclusion, it should be again emphasized that the above taxonomic discussion is based on our present incomplete knowledge of biological and morphological properties, and that it is subject to revision with the introduction of new data. Many points are left unsettled. For example, one might ask why rickettsiae could not equally well be regarded as protozoa. The distinction between bacteria and protozoa is made largely on morphological grounds, and the rickettsiae are morphologically closer to bacteria than to protozoa. They lack the internal structure commonly associated with protozoa, and are smaller than organisms classed as protozoa, with the possible exception of certain piroplasmas.

The difficulties encountered in any attempt to classify the viruses are even greater than in the case of the rickettsiae, and much work must be done before the former can be classified, even tentatively, in a reasonably satisfactory way.

The elementary bodies of psittacosis are often referred to as L.C.L. (Levinthal-Cole-Lillie) bodies, but Yanamura and Meyer (38) have recently pointed out that there is no justification for substituting this name for that originally given by Levinthal, namely "*Microbacterium multiforme psittacosis*." Similarly, Goodpasture (39) has proposed the name "*Borrelia variolae hominis*" for the elementary bodies of smallpox. The application of these specific names to the bodies in question indicates a belief that they, like the rickettsiae, are living morphological entities. In the course of time, the true relationship between such bodies as these and intracellular microorganisms such as the rickettsiae will probably become clear. As a matter of convenience, it seems justifiable to give generic, specific, and variety names to such pathogenic agents, even though their family and order relationships are not yet clear.

In discussing the properties of the pathogenic rickettsiae, certain principles will be established first by a detailed consideration of *Rickettsia prowazeki*, the cause of typhus; and the known differences between the other organisms and the type species will be pointed out in the discussion of each.

## RICKETTSIA PROWAZEKI (TYPHUS FEVER)

*Etiological studies.* As mentioned above, the etiological agent of typhus fever was first described by Ricketts and Wilder in 1910 (6) in lice fed upon typhus patients. Further evidence that the organism described by Ricketts is etiologically related to typhus was furnished by da Rocha-Lima (4) who named the organism *Rickettsia prowazeki*. The work of Wolbach, Todd and Palfrey (7), carried out under ideal experimental conditions, entirely confirmed the etiological concept previously formulated by Ricketts and da Rocha-Lima. Wolbach and his co-workers showed that lice, originally free from rickettsiae, acquired numerous rickettsiae which distended the intestinal lining cells, when fed on typhus patients. These workers also demonstrated intracellular rickettsiae in the lesions of man and experimental animals. When the intestines of lice, containing intracellular rickettsiae, were emulsified and injected into guinea pigs, typhus fever resulted, whereas injection of the intestines of rickettsia-free stock lice produced no illness. Considering the intestinal tract of the louse as the equivalent of a bacteriological culture medium, it could be said at this time that, in a sense, Koch's laws had been fulfilled.

In spite of the conclusive nature of the above described work, skepticism regarding the etiology of typhus persisted for many years. This was due partly to the fact that the experimental work was of such a nature that it could not be readily repeated, and partly to the difficulty of demonstrating *R. prowazeki* in mammalian tissues. These organisms were never demonstrable in large numbers, and, because of their small size and slight affinity for aniline dyes, could be demonstrated only in perfectly fixed and stained preparations, studied with great patience.

Neill (40) found that in guinea pigs reacting to Mexican typhus after intra-peritoneal inoculation, an acute fibrinous exudate formed in the scrotal sac, while the general peritoneal cavity showed no such reaction. Later, Mooser (41) found that many cells distended with rickettsiae were constantly present in this exudate. The free growth of the organism apparently depends upon the lower temperature obtaining in the scrotal sac. With the advent of this new rickettsia-rich material to work with, and of better methods for staining rickettsiae in sections, progress was more rapid.

Further confirmatory evidence of the etiologic relationship of *R. prowazeki* to typhus has been furnished by the cultivation of the organism within living or surviving cells in artificial media of various types (see below). In plasma tissue cultures, not only was the presence of rickettsiae accurately correlated with infectivity, but the incubation period of the induced infection was found to be roughly proportional to the number of organisms present in the cultures, ranging from 48 hours up to 25 days (42). Zinsser and Castaneda (43) showed that rickettsiae, freed from cells and washed repeatedly at the centrifuge, were still capable of producing typhus. On the whole, it seems safe to state that the etiological relationship of *R. prowazeki* to typhus is as well established as, for example, that of the tubercle bacillus to tuberculosis.

The purely theoretical concept that the etiologic agent of typhus may exist in three forms, the bacterial, the rickettsial, and the invisible, will be found frequently in early literature, and has even been sponsored more recently by such authority as that of Nicolle (44). The bacterium most commonly listed is *Proteus vulgaris*. The process of "evolution" devised as an explanation of the variation in form does more credit to the imagination of its proponents than to their critical judgment. The etiologic relationship of certain strains of *P. vulgaris* to typhus was naturally suggested by the high titer in which it agglutinated by typhus serum (see below). Claims that strains of *P. vulgaris* or other organisms cultivated on bacteriologic media have produced typhus fever in guinea pigs do not stand critical analysis.

The contention that the etiologic agent of typhus may exist in an invisible form is somewhat more difficult to disprove. A similar concept has been entertained for spotted fever (45). In no instance, however, has any direct proof for such a theory been advanced. The fact that material containing no demonstrable rickettsiae may be infectious is evidence of an inconclusive nature, since a few dozen rickettsiae in a cubic centimeter of fluid would be practically impossible to find and recognize. In the author's work with Hass (42), typhus tissue cultures were bisected, one half being embedded and sectioned for microscopic study and the other half injected into guinea pigs. In the great majority of cases, when infection occurred in guinea pigs as a result of the injection of one half of the culture, rickettsiae were found in paraffin sections of the other half. In a very few instances, cultures gave a positive inoculation test while no organisms were seen. In such instances, however, the incubation period of the induced infection was invariably greatly prolonged, suggesting that the number of organisms present may have been small enough to escape detection. These experiments indicated the improbability of invisible forms of typhus and spotted fever rickettsiae occurring under these conditions. The evidence for an invisible form of the etiologic agents of typhus and spotted fever under any other conditions is on the whole meagre and inconclusive.

*Size, filterability, morphology, and staining.* The smallest dimension of *R. prowazeki* is commonly given as 300  $m\mu$ , but forms which appear smaller than this and are almost on the borderline of visibility are occasionally seen, both within cells and outside of cells (freed by rupture of the cells). Even if we accept the figure of 300  $m\mu$ , these organisms would have approximately the same size as the elementary bodies of psittacosis (estimated at 250 to 275  $m\mu$ ). The latter are filterable, while rickettsiae are apparently non-filterable. Typhus rickettsiae rapidly lose virulence when freed from their host cells, and it is the author's opinion that the question of filterability needs further study under carefully controlled conditions. Isolated rickettsiae, freed from all remnants of cell cytoplasm and suspended in a medium in which they remained virulent for the duration of the experiment, would have to be employed for this purpose. So far as the author is aware, these conditions have not been satisfied. The rickettsiae of Q-fever, which appear somewhat larger than typhus rickettsiae, readily pass through Berkefeld N filters (46). The question of the filterability of

*R. prowazeki* is a matter of arbitrary definition and subject to some uncertainty, since the organism is obviously of such a size that it might, under certain conditions, prove filterable.

As seen in paraffin sections, *R. prowazeki* is always intracellular. In smears, extracellular organisms are often seen, as a result of the rupture of cells in the process of making the preparation. The organism exhibits considerable pleomorphism, but this factor has perhaps been over-emphasized. In its most characteristic form, it appears as a minute diplobacillus, each member of the diploid form averaging about  $0.6 \times 0.3 \mu$ . Diploid forms may at times appear as a single organism, because the space between the two organisms may not be seen. Short chains are not uncommon. Occasionally, in recently infected cells in tissue culture, long chains ranging up to  $40 \mu$  in length may be seen. Wobach and his co-workers (7) described similar forms in lice, in the early stages of infection "within non-swollen cells of the midgut at a time when very few cells are infected with the coccoid rickettsia." These long chains apparently develop under optimum conditions of nutrition only in the early stages of cell infection, and it seems unlikely that they represent a stage in a life cycle.

In very heavily infected cells, which may become greatly distended, the individual organisms appear as minute coccoid bodies, usually in diploid formation. These coccoid forms approach the limit of the resolving power of the ordinary microscope. That these minute forms are not an optical illusion, caused by the packing in the cells, is shown by the fact that they can occasionally be seen extracellularly when a distended cell has ruptured. Rather than to consider these forms as evidence of a life cycle, the author prefers to regard them, like the chain forms, as morphological variants dependent on nutritional factors. The possibility that variations in cellular immunity may be a factor in causing this pleomorphism, as has been suggested for the bodies of psittacosis (38), cannot be ruled out, of course.

*Rickettsia prowazeki* stains poorly with most aniline dyes. The organism is gram-negative in the sense that it cannot be recognized with certainty when stained by the gram method, and obviously does not retain the dye. In film preparations, the organism can be stained very satisfactorily by the Giemsa method, or by Macchiavello's modification of Cantaneda's stain (47). In the former method, the rickettsiae are stained blue or purple, while the cell cytoplasm is usually a pale blue. By the latter method, the rickettsiae are stained red and stand out sharply against a blue cytoplasmic background. In paraffin sections, the Giemsa method, after Regaud's fixation (48), stains the organisms very clearly, but the use of this method requires some experience.

The faint staining of the organism, particularly as applied to sections, has been exaggerated. It is quite true that in Giemsa-stained films rickettsiae are commonly pale as compared to staphylococci, for example, but in paraffin sections they may be stained by various methods as deeply as may be desired, and when overstained their apparent size is greatly exaggerated.

*Cultivation, and growth requirements.* Typhus rickettsiae have not been cultivated in the absence of cells. They grow freely in the various media containing

living or surviving cells which have been used for virus cultivation, namely, plasma tissue cultures, the Maitland medium, and the Zinsser-Wei-FitzPatrick medium.

Experiments by Wolbach and others (49) showed that *R. prowazeki* could multiply in a restricted way in plasma tissue cultures of mammalian cells at 37.5 C. Massive infection was not obtained in this early work. Nigg and Landsteiner (50) reported the unrestricted intracellular multiplication of the organism in a modified Maitland medium, consisting of minced guinea pig tunica vaginalis suspended in a mixture of serum and Tyrode's solution. Pinkerton and Hass (42, 51) reported similar massive multiplication of the organism in plasma tissue cultures grown at 32 C. In the Nigg-Landsteiner medium, multiplication was equally good at 32 or 37.5 C, but in the plasma tissue cultures the lower temperature was found to be essential.

The Zinsser-Wei-FitzPatrick medium (52) is essentially a modified Maitland medium, the serum-Tyrode mixture being solidified by adding agar, and the tissue (chopped mouse or chicken embryo) spread on the surface. The tissue fragments are more thoroughly bathed by the fluids in this medium, and the method represents an important improvement over previous similar methods.

In smear preparations from cultures of the above types, one finds large numbers of extracellular organisms, suggesting extracellular multiplication. When identical preparations are studied in paraffin sections, however, the evidence is clear that multiplication is intracellular, and that extracellular organisms have recently been set free by the rupture of distended cells during the preparation of the film. The contents of a single cell, packed and distended with rickettsiae, may cover a large area if the organisms were spread out only one layer deep, much as a minute drop of blood will make a film over the entire surface of a coverslip.

Although their growth requirements are in general similar to those of the typical viruses, an interesting difference has been brought to light. The author found that in plasma tissue cultures typhus rickettsiae died rapidly at 37 C, and grew best at 32 C (53). This behavior is probably to be correlated with the fact that certain strains, after intraperitoneal injection in the guinea pig, grow more readily in the scrotal sac, where the temperature is lower, than in the general peritoneal cavity, and produce in the scrotal sac a fibrinous exudate containing large numbers of heavily infected cells (the Neill-Mooser reaction described above.) In the Maitland medium, rickettsiae multiply equally well at 32 C and 37.5 C. This apparent discrepancy is probably to be explained on the basis of the different conditions obtaining in the Maitland medium and in plasma tissue cultures. In the former, the cells are surviving rather than actually living, and mitotic division of the cells is rarely seen, while in plasma tissue cultures the cells multiply rapidly and the metabolic rate of the cells is probably higher. From these facts the conclusion was drawn (54) that typhus rickettsiae grow best in cells which are metabolizing slowly. Zinsser (55) has recently confirmed this and pointed out that certain viruses, in contrast to rickettsiae, grow best in healthy, actively metabolizing cells.

Still further confirmation has come from the observation by the author with

Bessey (56) that riboflavin deficiency, a condition which lowers intracellular metabolism by interference with the formation of the yellow respiratory enzyme, causes marked loss of resistance to typhus infection in the rat, allowing the rickettsiae to multiply profusely in the reticulo-endothelial cells in various organs, and resulting in a fatal disease. A similar specific lowering of resistance to psittacosis as a result of thiamin deficiency has been reported by the author with Swank (57). A smaller, and presumably more perfectly adapted virus, however, (the Lansing strain of poliomyelitis), has been found (58) to cause equally severe illness in normal and riboflavin-deficient mice, with an apparently higher mortality rate and somewhat earlier death in the normal than in the deficient animals. Similarly, the Rous chicken sarcoma grows much more slowly in riboflavin-deficient than in normal chickens, the deficient birds outliving the controls by several weeks (38). Thus it appears that certain viruses, in contrast to typhus rickettsiae, are inhibited by riboflavin deficiency in their host.

Study and speculation have led various workers to regard intracellular parasitism as primarily an adaptation to intracellular existence, associated with an acquired ability to utilize intracellular enzymes of the host cell, and with a corresponding loss (by disuse?) of enzymes on the part of the parasite. In a general way, but not necessarily with any degree of accuracy, this loss of enzymatic complexity may be correlated with a diminution in size of the parasites. From this point of view the rickettsiae are of particular interest, since they are intermediate in size between most free-living bacteria and the smaller viruses, and, from the above observations regarding their growth requirements, might also be regarded as intermediate in their degree of adaptation to intracellular conditions, since they grow best in cells which are metabolizing slowly.

Further study is needed, however, before we can safely generalize regarding the metabolic activity of the viruses. It is not inconceivable, for example, that some of the smaller viruses may be shown to multiply most freely in slowly metabolizing cells, or even to approach the free-living bacteria in their metabolic requirements. On the other hand, it is of interest to note that a number of protozoa, for example "toxoplasma," although large in size and complex in structure, behave in mammalian tissues as obligate intracellular parasites (59).

The absence of a single important metabolic activator might be as effective in preventing the cultivation of an organism in cell-free media as the absence of a number of enzymes. The problem in each intracellular parasite, is to find out exactly what activators are lacking. Presumably, if the missing enzymes or other activators could be supplied, together with suitable food substances and physical conditions, any obligate intracellular parasite could be cultured without living cells. It should also be pointed out, however, that parasites within cells may be protected from certain toxic substances which prevent their multiplication outside of cells.

The study of rickettsiae in plasma tissue culture affords a logical approach to the question of their growth requirements, both physical and metabolic.

As mentioned above, typhus rickettsiae disappear rapidly from their host cells in tissue cultures maintained at 37.5 C, and the cultures are no longer

infectious for guinea pigs after about the tenth day. At 42 C, the rickettsiae disappear even more rapidly from the cells. At 27 C, the cells die in about two weeks, and the rickettsiae disappear. At 32 C, however, the rickettsiae find optimum conditions for intracellular growth, and practically every cell becomes distended with them (53). These heavily parasitized cells are apparently uninjured and are still capable of mitotic division. Such cultures remain heavily infected for several months when maintained at 32 C, but when placed at higher temperatures rapidly become free of rickettsiae and lose their infectivity. This striking dependence on temperature is probably indirect rather than direct. The organisms grow luxuriantly at 32 C not because they prefer that temperature, but only because that temperature is most effective in bringing about suitable growth conditions within their host cells. These growth conditions, as in the case of riboflavin deficiency, probably result from a lowering of the intracellular metabolism.

Alterations in the partial pressure of oxygen in such cultures have been shown to be without effect on the intracellular growth of typhus rickettsiae, unless the concentration of oxygen becomes so low that the cells die. In this case, the rickettsiae also soon die, since they are unable to live within dead cells. Similarly, alterations in the pH of the tissue culture medium are without effect except insofar as they may cause the death of the cells.

It is probable that such alterations in pH of the medium do not appreciably change the intracellular pH. Attempts to alter the pH of the cell cytoplasm by organic acids and bases ( $\text{CO}_2$  and  $\text{NH}_3$ ) were, however, likewise without apparent effect on the intracellular multiplication of rickettsiae (60).

Typhus rickettsiae in tissue culture not only fail to multiply extracellularly in the plasma clot, but apparently survive only for brief periods when set free from disintegrating cells. This statement is based on the fact that, even in cultures where cells are allowed to disintegrate, organisms are seen in the plasma clot only in extremely small numbers, and never at a great distance from their host cells (51).

Another method of approach to the problem of the metabolism of rickettsiae is represented by attempts to cultivate them in symbiosis with free-living bacteria, either in direct contact with these organisms or near enough, on solid media, to permit the diffusion of nutrient substances, on the theory that the enzyme systems of the free-living organisms might be utilized in some way by the rickettsiae. Although the possibilities have by no means been exhausted, a considerable number of experiments of this type have been entirely negative.

On the whole, then, it can be said that little definite information is available regarding the actual metabolism of rickettsiae and other intracellular parasites, although the rickettsiae seem to be particularly suitable for the study of this problem.

*Cytological studies.* Rickettsiae have the advantage over the smaller viruses as objects for cytological study of being clearly visible as individuals in their host cells. They can be clearly seen in unstained living cells with either dark or light field illumination, and their behavior under various conditions can be

readily observed. In the early stages of their intracellular multiplication, typhus rickettsiae lie motionless in an apparently gelatinous cytoplasm, surrounded by only a narrow clear halo. Later, the organisms are grouped in what appear to be fluid vacuoles and move freely within these vacuoles by Brownian movement (60). True motility has not been observed, either within or outside of cells. As infected cells disintegrate, small groups of rickettsiae, moving freely within these small spherical fluid vacuoles, are set free and float out into the surrounding medium. This mechanism is probably important in the spread of infection from cell to cell.

Typhus rickettsiae multiply exclusively in the cytoplasm of cells in tissue cultures, and are never seen in nuclei. This behavior is in striking contrast to that of spotted fever rickettsiae (61). Since typhus rickettsiae would, like spotted fever rickettsiae, probably be occasionally included within nuclei, as the nuclear membrane is reformed after mitotic division, one can only conclude that conditions within the cell nuclei are for some reason incompatible with the life and growth of typhus rickettsiae. The reason for this difference in behavior between the two organisms has not been ascertained.

The behavior of typhus rickettsiae during mitotic division of their host cells is interesting. The organisms are markedly reduced in number during this process, and tend to collect at the poles of each cell (51). They also, in many instances, assume a more spherical shape. The period of mitotic division, perhaps because of the increased metabolic rate within the cells, is apparently unfavorable for the rickettsiae, and many of them die at this time. Those which survive, however, rapidly multiply until they distend the daughter cells.

When fat droplets appear within cells in tissue culture, the rickettsiae are never seen within these droplets, but they crowd the cytoplasm around the droplets.

The possible relationship of rickettsiae to mitochondria has frequently been a subject for speculation. Their morphology and staining reactions are not inconsistent with such a viewpoint, but Cowdry and Nicholson (62), after staining mitochondria and rickettsiae alternately in mammalian cells, concluded that the two types of structures were separate and distinct. The fact that rickettsiae may distend cells, and in the case of spotted fever even multiply within nuclei where mitochondria have never been described, is also evidence of their different nature. It seems quite clearly established that rickettsiae, as seen in parasitized mammalian cells, are unrelated to the mitochondria of these cells. In view of the similarity of certain rickettsiae to the intracellular symbionts of insect tissues and the lack of a sharp line of demarcation between the latter and mitochondria, the question of a relationship between rickettsiae and the mitochondria of insect tissues should perhaps not be considered as finally settled. Although it would be entirely possible to do so, no one has actually watched rickettsiae enter cells and observed their actual multiplication after entering. By this simple observation, the question of their intracellular origin could be definitely settled.

*Viability under various environmental conditions.* *R. prowazeki* is readily killed by heat, drying, or chemical agents. A few minutes at 50 C suffice to destroy the virulence of blood and tissue emulsions, while such materials when dried under ordinary conditions at room or incubator temperatures become non-virulent in a few hours. Blanc and Baltazard report, however, that virulence is retained in the thoroughly dried feces of infected fleas for periods up to 651 days.

Any discussion of the viability of the organisms must take into consideration the medium and the protective action of the cytoplasm of the host cells. As has been brought out above, rickettsiae appear, under all conditions in which they have been studied, to survive for only a few hours when freed from this protection. The supernatant fluid from heavily infected Maitland cultures of typhus rickettsiae is non-virulent when injected into guinea pigs. In defibrinated typhus blood, virulence is lost in about 72 hours at incubator temperatures and in less than four days at room temperature. Scrotal sac exudate, containing many intracellular typhus rickettsiae, was found by the author (63) to lose virulence between the third and eighth days when suspended in glucose broth at room temperature.

Rickettsiae are commonly said to be, like many of the viruses, resistant to the action of glycerine. Strictly speaking, however, it is probably not a question of resistance to this substance, since the glycerine probably does not come in contact with the intracellular organisms. Glycerine probably helps to preserve the viability of the organisms by virtue of its dehydrating action on their host cells. Infective typhus guinea pig tissues, suspended in glycerine, can retain their virulence for several months at 0 C (63), while the same tissues suspended in physiological salt solution or merely sealed in test tubes lose their virulence in a few days under these conditions.

At -20 C, however, a brain or spleen from a typhus guinea pig, merely sealed in a test tube, retains its virulence with a gradual loss of titre for periods ranging up to eight months (63). Scrotal sac exudate, minced and suspended in Tyrode's solution, becomes non-virulent in two weeks at -20 C, but if suspended in a mixture of equal parts of blood serum and Tyrode's it retains virulence for periods up to six months (63). Topping (64) finds that typhus rickettsiae remain virulent for several months (end-point not determined) in tissue dried in the frozen state in the "lyophile" form or by the "Cryochem" process.

Nigg (65) has reported that typhus rickettsiae in sealed Maitland cultures at 37 C are virulent for periods ranging up to several months. Although the host cells undoubtedly die under these conditions, they do not disintegrate and apparently continue to maintain the rickettsiae in a viable state. As has been said, defibrinated blood from a typhus guinea pig loses its virulence in a few days at incubator temperatures.

On the whole, the behavior of typhus rickettsiae under various environmental conditions is similar to that of many viruses. In both cases, the environmental conditions which preserve virulence are probably those which tend to maintain the integrity of the cytoplasm of the host cells. The conditions under which extracellular rickettsiae retain their virulence have not been sufficiently studied.

*Pathology, and pathogenicity for mammals.* The histopathology of typhus infection need not be discussed in detail here. Suffice it to say that the characteristic pathologic changes are brought about by the multiplication of *R. prowazeki* within the endothelial cells of the small blood vessels throughout the body, but particularly in the skin and brain. The pathology is entirely similar in man and in experimental animals.

Among the lower animals, monkeys, guinea pigs, dogs, cats, rabbits, rats, mice, gophers, woodchucks, jackasses, ground squirrels, and flying squirrels are susceptible in varying degrees to typhus infection. In many of these animals, no fever or other outward manifestation of infection is seen, but the infection persists for long periods (up to 370 days in white rats) in an inapparent form.

The most satisfactory animal for the laboratory study of typhus is the guinea pig, in which both murine and human typhus causes a febrile but non-fatal illness. The differences in the scrotal reaction which are of importance in distinguishing between the two strains of typhus, will be discussed below. The scrotal reaction occurs only after intraperitoneal injection, but a febrile illness and the characteristic focal brain lesions are produced equally well by subcutaneous inoculation.

*Strain variation.* An outstanding characteristic of the pathogenic rickettsiae is their tendency to occur in strains of widely different virulence, both for man and for experimental animals. The possible relation of the pathogenic rickettsiae to certain of the non-pathogenic rickettsiae in arthropods requires further study, but the evidence at present is against any close relationship (13).

In addition to strain variations in virulence, however, careful study has brought to light interesting biological variations between strains of *R. prowazeki*. These differences are more or less permanent modifications, probably caused by prolonged residence in different species of arthropods and in different species of mammals.

Two distinct types of *R. prowazeki* are recognized. The differences between these two types were believed by the author (18) to be of sub-specific magnitude, and consequently the two types may be given the variety names *R. prowazeki* var. *prowazeki* and *R. prowazeki* var. *mooseri*. The former is the etiological agent of louse-borne human typhus, of the type which has occurred in great epidemics on the European continent and has been called European or epidemic typhus. *R. prowazeki* var. *mooseri* is the name applied to the etiological agent of a somewhat milder, usually endemic, form of the disease occurring in various parts of the world, and apparently enzootic in rats. This form of the disease is carried from rat to rat by the rat louse (and by the rat flea) and from rat to man by the rat flea. It is best called murine typhus, a name suggesting the importance of the rat in its epidemiology. According to Zinsser, both forms of the disease may occur in epidemic or endemic form, and the murine type in Mexico can, like the human type, be carried from man to man by lice.

The differences between these two varieties of *R. prowazeki* may be summarized as follows:

1. *R. prowazeki* var. *mooseri* after intraperitoneal injection in rats causes a febrile illness, while *R. prowazeki* var. *prowazeki* produces only an inapparent

infection. Male rats infected with *R. prowazeki* var. *mooseri* suffer a mild inflammation of the tunica vaginalis with many serosal cells laden with rickettsiae while *R. prowazeki* var. *prowazeki* causes no such reaction.

2. *R. prowazeki* var. *mooseri*, when introduced *per anum* into *Pediculus humanus*, kills this arthropod in a few days, while *R. prowazeki* var. *prowazeki* requires about three weeks. This phenomenon is probably independent of the number of viable organisms injected.

3. After intraperitoneal injection in male guinea pigs, *R. prowazeki* var. *mooseri* almost constantly induces an acute inflammation of the scrotal sac with numerous visible organisms, while *R. prowazeki* var. *prowazeki* either fails to cause such a reaction at all or produces it irregularly and in a milder, more transient form (66).

4. Zinsser's studies with Castaneda (67) brought out certain minor immunological differences between the two varieties, which will be discussed below.

5. Perhaps most important of all distinguishing features from a practical point of view is the fact that *R. prowazeki* var. *prowazeki* grows much less luxuriantly, not only in living experimental animals with resistance reduced by various methods, but also in the various media containing living or surviving cells. This fact makes the production of vaccine, in practical quantities, a more difficult problem than in the case of *R. prowazeki* var. *mooseri*. Further discussion of this phenomenon with reference to immunological problems will be found below.

These differences between the two varieties of *R. prowazeki* seem at first glance to be relatively trivial, but careful study has shown that they are constant and important. Nobody has succeeded in changing one variety into the other, even after years of passage in different species of animals. Human typhus rickettsiae, which have been producing only an inconspicuous scrotal reaction in guinea pigs, can be made to produce a severe scrotal reaction, comparable to that seen in murine typhus, by passing the infection through rats and then back to guinea pigs (68). In subsequent transfers, however, the scrotal reaction reverts to its mild, inconspicuous type. It is, however, believed, without anything like conclusive proof, that with crowding together of human beings and an abundance of lice, the severe, epidemic infection with *R. prowazeki* var. *prowazeki* may evolve in months or years from a single case of human infection with *R. prowazeki* var. *mooseri* acquired directly from a rat by the bite of a rat flea. There is, on the other hand, some reason for believing that *R. prowazeki* var. *prowazeki* may be endemic in man in certain parts of the world, and if so, important epidemics may start directly, without the necessity of a process of transformation of one variety into the other.

Zinsser (69) has introduced strong evidence that cases of typhus in the North Atlantic states are late recrudescences of childhood infection with *R. prowazeki* var. *prowazeki*, acquired in Europe. If this be the case, the survival of *R. prowazeki* var. *prowazeki* during interepidemic periods, quite independent of the murine form of the disease, could readily be explained.

*Immunology.* Infection with the rickettsial diseases usually confers long-

lasting immunity. The study of the problem of active immunization in typhus and spotted fever has centered about the development of methods for the accumulation of rickettsiae in sufficiently large concentration to be of practical value. Ordinarily, the concentration of rickettsiae in the tissues of infected laboratory animals is too low to be of value. Tissue vaccine, therefore, of the type made by Laidlaw and Duncan for immunization against distemper, is of no value.

Weigl (70) took the first step in 1930 toward a solution of this problem by using the heavily parasitized intestinal tissues of lice, infected by introducing the virus *per anum*, and subsequently maintained on typhus-immune human beings. Although of proven value, this method of vaccine production cannot be utilized on a large scale because it is too time-consuming.

Benzol poisoning, perhaps because of its destructive effect on the bone marrow, was found by Zinsser and Castaneda to increase the number of rickettsiae in the peritoneal lining cells of rats infected intraperitoneally with murine typhus. Better and more constant results were later obtained by subjecting the rats to suitable doses of x-rays (71). Probably because of the low natural susceptibility of the rat, this method is not effective for human typhus. The "agar tissue culture" method of Zinsser and co-workers has already been described.

*R. prowazeki* was grown on the chorio-allantoic membrane of the developing chick embryo by Zia (72). The concentration of rickettsiae obtained by this method was not great enough, however, to justify its use for vaccine production. Cox showed that much higher concentrations of rickettsiae could be obtained by injecting the organism into the yolk sac of the developing egg embryo (73). Smears of such preparations showed such large numbers of apparently extracellular organisms that for a time it was believed that extracellular multiplication was taking place. It is now known, however, that multiplication is entirely within the lining cells of the yolk sac, and that here, as under all other known conditions, *R. prowazeki* behaves as an obligate intracellular parasite.

The Zinsser-Wei-FitzPatrick medium (52) and the method of Cox both have the advantage over other methods of being applicable to human typhus (*R. prowazeki* var. *prowazeki*) and spotted fever (*D. rickettsi*) as well as to murine typhus.

The author, with Bessey (56) obtained large concentrations of *R. prowazeki* var. *mooseri* by the intraperitoneal injection of murine typhus rickettsiae in riboflavin-deficient rats. The possibilities of this method for vaccine production have not been exploited. No success was achieved by this method with either human typhus or spotted fever rickettsiae, probably because of the high natural resistance of the rat to these agents. It is possible that the adaptation of this method to guinea pigs, which are highly susceptible to human typhus and spotted fever, might give results of practical value.

Castaneda (74) has discovered still another method for producing high concentrations of *R. prowazeki* var. *mooseri*, namely, the intratracheal injection of the virus in rats and mice. He believes that the murine strain is antigenically

broader than the human, and that successful vaccination against the latter can be achieved by the former if large doses are given. If this proves to be so, the problem will be simplified, since high concentrations of *R. prowazeki* var. *mooseri* are relatively easily obtained by all known methods.

Further work is necessary to decide on the relative merits of the various ingenious methods of vaccine production outlined above, but at present the yolk sac method of Cox has probably the greatest demonstrated practical value and is being utilized most extensively. Even in the face of continued failure to cultivate typhus rickettsiae on bacteriologic media, the outlook for large-scale vaccine production is by no means dark.

*Passive immunization.* By injecting very large quantities of rickettsiae into a horse over long periods of time, Zinsser and Castaneda (75) have prepared an immune serum of considerable potency for guinea pigs infected with both human and murine typhus. The practical value of such serum for human patients has not been demonstrated.

*Living virus immunization.* Laigret and co-workers (76) have developed a method of immunization based on the injection of guinea pig tissues containing the infective agent of murine typhus in an attenuated form. Such methods are effective only insofar as they introduce living rickettsiae and cause actual infection. The dangers of such procedures are obvious, and a few fatalities have occurred from their use in Chile. It cannot be denied, however, that under certain conditions, as in the face of devastating epidemics, the production of the relatively mild murine typhus in the population on a large scale might be a justifiable emergency measure. Experiences during the present war will no doubt give important information regarding the value of various prophylactic measures.

Vaccination with mixtures of living rickettsiae and immune serum (Zinsser and Macchiavello, (77)) should also be mentioned in this connection. The difficulty of this method is in controlling accurately the balance between the serum and the living organisms.

*Agglutination reactions.* Although the agglutination of certain strains of *Proteus vulgaris* by sera of patients infected with typhus and spotted fever has been used for many years as a diagnostic procedure, no true biological relationship between that bacterium and the rickettsiae has been demonstrated. Castaneda (78) believes that the phenomenon depends on the possession of a common carbohydrate antigen by the two organisms. The titre of agglutination is unusually high for a non-specific reaction, but, however closely related the two organisms may be antigenically, they have in their present forms nothing in common except the probable possession of a common antigen. Uncritical experiments suggesting that *P. vulgaris* may cause typhus fever are not worthy of serious consideration.

In typhus, agglutination is usually found in relatively high titre to the OX 19 strain of *P. vulgaris*, and in low titre with the OX K strain. This fact is of value in differentiating typhus from *tsutsugamuchi* disease, since in the latter the agglutination titre is characteristically high with OX K and low with OX 19.

In spotted fever, agglutination in relatively low titre with both OX K and OX 19 is the rule (18).

Agglutination tests can also be carried out with suspensions of *R. prowazeki* of both murine and human varieties, obtained and concentrated by any of the methods described above. Cross agglutination tests carried out by Zinsser and Castaneda (67) showed much higher agglutination titres with the homologous rickettsiae than with the rickettsiae of the other strain. Immune serum also protected better against the homologous infection.

Complement fixation tests have been shown to be positive in typhus (79).

*Latent infection.* On the whole, the tendency of the rickettsiae to cause latent infection is somewhat less impressive than that of many viruses. The brains of rats may harbor the rickettsiae of typhus for as long as 370 days (80). The evidence introduced by Zinsser (69), although strongly suggesting that latent infection may persist in man for many years, should probably not be accepted without further confirmation. Attempts to prove that thromboangiitis obliterans is a late manifestation of typhus (81) deserve only casual mention, since no valid evidence to support this view has been introduced.

*Chemotherapy.* Kikuth (82) has recently stated, but without specific data, that the sulfonamides exert a definite chemotherapeutic effect in rickettsial diseases. Topping (83) obtained negative and even suggestively detrimental effects in typhus-infected guinea pigs by the administration of prontosil and sulfapyridine. Chemotherapy seems as yet to offer little promise, and its use in typhus may even be dangerous.

*Methods of diagnosis.* Sporadic cases of rickettsial disease are of frequent occurrence in various parts of the world. Many of these cases are clinically so mild or atypical that special laboratory tests are necessary to establish a diagnosis. In the majority of such cases, the intraperitoneal injection of 5 ml. of the patient's blood into each of two guinea pigs during the first few days of illness will result in a febrile disease with involvement of the scrotum or scrotal sac. This phenomenon, which is the result of rickettsial growth in a region of lower temperature, is almost diagnostic of typhus or spotted fever. By killing a guinea pig and making smears from the scrotal sac, a presumptive diagnosis can usually be made, since in typhus we find cells packed with rickettsiae, while in spotted fever the organisms are fewer and larger, and tend to have the characteristic lanceolate form already described. Conclusive proof of the nature of the infection is usually obtainable by carrying out cross-immunity tests with known strains of typhus and spotted fever rickettsiae.

In certain cases, very mild strains may be encountered, which do not become manifest until transfers are made to a second series of guinea pigs. Certain strains may even be difficult to maintain in guinea pigs and cross-immunity tests may be ambiguous. Tissue culture studies, with the application of the criterion of intranuclear *versus* intracytoplasmic growth (18) may be necessary to establish a diagnosis in such cases.

In order to establish a diagnosis during convalescence cross-protection tests with the patient's serum may be carried out. The various diagnostic methods

have been described in detail by the author (18). Animal inoculation is a simple procedure and should be utilized more frequently than it has been in the past.

#### DERMACENTROXENUS RICKETTSI (SPOTTED FEVER)

*Dermacentroxenus rickettsi* is the etiological agent of a number of diseases of the spotted fever group. Although similar in many respects to *Rickettsia prowazeki*, important differences exist which fully justify its assignment by Wolbach (17) to a different genus. All diseases of this group are transmitted by various species of ticks. Originally, the disease was believed to be sharply localized to the general region of the Rocky Mountains, and was called Rocky Mountain spotted fever. It has now been demonstrated that essentially similar diseases, often showing minor clinical variation and transmitted by different species of ticks, occur in nearly all parts of the world.

*Etiological studies.* The development of our knowledge of the etiology of spotted fever parallels in many ways that of our knowledge of the etiology of typhus. Ricketts (84), and King (85), independently announced in 1906 the transmission of spotted fever to guinea pigs by ticks. Ricketts also demonstrated the hereditary transmission of the infection in ticks. He undoubtedly saw the etiological agent of the disease in the tick but did not differentiate it clearly from non-pathogenic organisms of similar appearance. He noted, however, the occurrence of large numbers of organisms in the ova of ticks, and showed that these organisms were agglutinated by immune serum from human beings and guinea pigs. Ricketts also described the organism, which he likened to the influenza bacillus in appearance, in blood smears from guinea pigs and man, but this observation has never been confirmed.

Wolbach (17) published in 1919 the results of his thorough and careful etiological and pathological studies, and named the etiological agent *Dermacentroxenus rickettsi*. Wolbach differentiated between this organism and non-pathogenic organisms in ticks, and was the first to demonstrate the intranuclear multiplication of the organism in tick tissues.

Confirmatory evidence of the etiological relationship of the organism to the disease was furnished by its cultivation in tissue culture. In mammalian cells grown *in vitro*, the presence of the organism is accurately correlated with infectivity (61). The same is true of infected tick tissues, but here, except for the intranuclear localization of the organism, it is difficult to distinguish it from non-pathogenic organisms (13). In tissue cultures initiated from the tissues of infected guinea pigs, this difficulty does not arise.

Parker (45) believed that the etiological agent of spotted fever could occur in an invisible form, and that the "virus" could exist in ticks in an inactive form which may become active when the ticks ingest blood. He has found high titres of infectivity in ticks free from rickettsiae demonstrable by smears, and has found rickettsiae in ticks which were not infective. It seems probable that rickettsiae in non-infective ticks are the non-pathogenic rickettsiae which are often found in ticks, and which may closely resemble *D. rickettsi* morpho-

logically. These non-pathogenic rickettsiae are never found within cell nuclei, however, and are believed to be distinct from *D. rickettsi*. The failure to find rickettsiae in smears of infective ticks may be explained by the fact that the smear method does not demonstrate intranuclear forms. In paraffin sections, cytoplasmic rickettsiae may be absent while thousands of nuclei contain many rickettsi. In tissue cultures, infectivity was never found in the absence of demonstrable rickettsiae. It is the author's opinion that all the observed phenomena can be explained without assuming the existence of the etiological agent of spotted fever in an invisible form.

Parker's observation (45) that hibernating ticks contain the etiological agent of spotted fever in a non-infective or merely immunizing form, which changes to a highly virulent form when these ticks are fed, has never been satisfactorily explained. It has not been established, however, that infection depends on factors other than the concentration of viable rickettsiae.

*Size, filterability, morphology, and staining.* The smallest dimensions of *D. rickettsi* are about the same as those of *R. prowazeki*, and material passed through Berkefeld filters has never been shown to be infectious. The determination of size by filtration experiments, in addition to the usual uncertainties of this procedure, is made difficult by the fact that the organisms, when freed from cells, rapidly become non-virulent unless suitable physical conditions are provided. Wolbach (17) found that thoroughly crushed tissues of infected ticks, suspended in salt solution, were non-virulent.

*D. rickettsi*, under certain conditions, shows a striking morphological resemblance to *R. prowazeki*. As seen in the scrotal sac of infected guinea pigs, differentiation on morphological grounds is extremely difficult. The most definite difference is the occurrence in spotted fever of paired forms, the individual members of which have tapered ends, so that they somewhat resemble small pneumococci. This form was first described by Wolbach (17) and was believed by him to be a resting stage, while the bacillary forms, of which he described a large and a small form, were believed to be multiplicative stages.

As seen in tissue cultures (61, 54), where an excellent opportunity is afforded for morphological study owing to the heavy infection and perfect fixation of the small tissue fragments, the morphological range of *D. rickettsi* is certainly greater than that of *R. prowazeki*. The large lanceolate forms at times reach the size of pneumococci. Frequently, in the same cell, the nucleus contains clusters of exceedingly minute paired granules which are resolved with difficulty, while the cytoplasm contains diplobacilli of average size, together with a few of the very large lanceolate forms. Except for the constant occurrence of these different forms in cultures known not to be contaminated, one would have difficulty in believing that they were forms of a single organism. Rarely, the large forms have been seen in nuclei, but never in nuclei which are distended with organisms. The significance of the large lanceolate form is not clear. It may, as Wolbach suggested, be a developmental stage (a resting, resistant form) or its occurrence may be determined simply by nutritional conditions. In any case, it is distinctive of *D. rickettsi*, and similar forms are not seen in typhus.

Long chains or apparently filamentous forms, though not seen by Wolbach in spotted fever, have been found in spotted fever tissue cultures and may be indistinguishable from the chain forms seen in typhus.

The staining characteristics of *D. rickettsi* are in general similar to those of *R. prowazeki*. The Giemsa method (48) and the Macchiavello method (47) both give excellent results in smears. *D. rickettsi* is rather more easily demonstrated in paraffin sections than *R. prowazeki* and may be stained by a wider variety of methods, including the ordinary eosin-methylene blue stain (under ideal conditions in thin sections). A modification of the Macchiavello method (38) has recently been found to stain the elementary bodies of psittacosis red, against a blue cytoplasmic background. This method was found in the author's laboratory to be unsatisfactory for staining spotted fever rickettsiae. There are two modifications of the Macchiavello method that stain *D. rickettsi* very sharply and deeply in sections of the guinea pig testicle. By the following modification, the organisms stain deep red and are clearly distinguishable, even though the surrounding tissue is also partly red.

1. Fix tissues in Regaud's fluid, cut thin paraffin sections and run through xylol and graded alcohols to distilled water in the usual way.

2. 1% aqueous methylene blue overnight.
3. Decolorize in 95% alcohol.
4. Counterstain in 0.25% aqueous basic fuchsin for 30 minutes.
5. Decolorize rapidly (about 3 seconds) in 0.5% citric acid.
6. Differentiate rapidly in absolute alcohol.
7. Clear in xylol and mount in gum dammar.

The second modification stains the rickettsiae deep blue against a background which is partly red and partly blue:

1. to 5. Procedures same as above.
6. Wash lightly in distilled water.
7. Counterstain again in 1% aqueous methylene blue for 5 seconds.
8. Differentiate in 95% alcohol.
9. Absolute alcohol and xylol. Mount in gum dammar.

It should also be noted that *D. rickettsi* may be stained fairly well by Goodpasture's method (86).

*Cultivation, and growth requirements.* In general, the conditions which permit the existence and multiplication of *D. rickettsi* are similar to those necessary for *R. prowazeki*. The latter in the louse is confined to the cells lining the intestinal tract, while *D. rickettsi* in the tick is found within practically every type of cell and in every organ. In mammalian tissues, *R. prowazeki* grows only in vascular endothelium and in the serosal cells lining the peritoneal cavity (87), while *D. rickettsi*, in addition to the above cell types, also grows in smooth muscle cells of arteriolar walls and in macrophages (17). This ability to utilize a wider variety of cells as hosts is of great interest, but its significance, in term of growth requirements, cannot be learned until we know the details of the metabolic processes in the various types of cells.

*D. rickettsi*, in guinea pigs, shows a preference for the scrotum and testes,

probably because of the lower temperature obtaining there, and in plasma tissue cultures grows best at 32 C. The effect of riboflavin-deficiency has not been carefully studied. Moderate numbers of spotted fever rickettsiae have been found by the author in the peritoneal cavity of riboflavin-deficient rats following intraperitoneal injection, in contrast to the absence of rickettsiae in rats similarly injected and on a normal diet, but the effect is much less striking than that seen in typhus. By producing satisfactory riboflavin deficiency in a more susceptible animal like the guinea pig, results of interest might be obtained.

In plasma tissue cultures, the restricted multiplication of *D. rickettsi* in the cytoplasm of cells and its unrestricted multiplication in nuclei (see below) should furnish a clue to its metabolic requirements. In spite of many attempts, it has been found impossible, by alterations in the temperature, pH, gaseous content, or chemical composition of the tissue culture medium, to produce marked intracytoplasmic multiplication of spotted fever rickettsiae, comparable to that seen with typhus rickettsiae (54).

In the yolk sac of the developing egg embryo (73) and in the Zinsser-WeitzPatrick medium, the large number of spotted fever rickettsiae seen in smear preparations suggests that the organisms grow more freely than they do by other methods. It would be of interest to study cultures of the above types in paraffin sections, in order to find out exactly what types of cells are invaded and how freely the organisms grow in the nucleus and cytoplasm of the infected cells.

*Viability under various environmental conditions.* Spotted fever rickettsiae are killed in a few minutes by exposure to moist heat at 50 C or to chemical agents, and in a few hours by thorough desiccation at room temperature. Infectious guinea pig blood retains its virulence at room temperature for only about a week, and the titre of infectivity diminishes rapidly during that period. At 5 C, virulence is maintained for somewhat longer periods, ranging up to fifteen days. At -7 C, brain and spleen from infected guinea pigs remain virulent, either when suspended in glycerine or when merely sealed in containers to prevent drying, for periods ranging up to a year (88). It is probable that retention of virulence for even longer periods of time, and with slower loss of titre could be obtained at -50 C.

The evidence obtained from paraffin sections of plasma tissue cultures indicates that *D. rickettsi* survives only for brief periods when set free by the disintegration of cells (61, 54). Extracellular rickettsiae are seen in the fibrin clot at the edge of the tissue fragments in somewhat larger numbers than are typhus rickettsiae, but are never present in such concentrations as to suggest their survival for more than a few hours.

*Cytological studies.* The striking feature of *D. rickettsi* in plasma tissue cultures is its apparent preference for the nuclei of cells, where it grows in compact clusters (61, 54, 18). At times, the entire nucleus becomes distended with organisms, and there is definite peripheral condensation of the nuclear chromatin, similar to that seen in association with the intranuclear inclusions of certain virus diseases. Occasionally, from one to five smaller spherical clusters

may be seen within a nucleus, and these clusters show a marked tendency to maintain a spherical form. In the cytoplasm of the cells, the rickettsiae are never in clusters, but grow sparsely and are arranged diffusely.

The occurrence of these spherical intranuclear clusters, which at times are composed of such minute elements that they appear to be finely granular, is naturally suggestive of the intranuclear inclusions seen in filterable virus infections. The resemblance is made still more striking by the fact that frequently the organisms forming the intranuclear clusters are stained bright red in Giemsa preparations, while the intracytoplasmic organisms stain blue. Nicolau (89) has reported that with certain stains the intranuclear inclusions of herpes simplex may be shown to be composed of compact masses of bacillary structures, and in his illustrations the picture is remarkably similar to that of intranuclear spotted fever rickettsiae. The author (90) studied the intranuclear inclusions of Virus III in tissue cultures, fixed and stained by the Regaud-Giemsa method, and was unable to demonstrate any internal structure. Whatever the nature of the herpes inclusions may be, it is unsafe to generalize regarding the nature of intranuclear inclusions in general, since it appears probable that their nature may vary (3) and that certain types of intranuclear inclusions may be caused by chemical injury.

Multiplication of parasites within the nuclei of their host cells is a very uncommon occurrence. Excluding "virus bodies" like those of herpes, the only definite microorganisms other than *D. rickettsi* which exhibit this phenomenon are relatively large protozoa, such as *Karyophagus salamandrae* (91) which is a parasite of the salamander.

The unique intranuclear localization of *D. rickettsi* was utilized in the classification of atypical strains which gave ambiguous cross-immunity reactions (18). Alexander and Mason (92) have recently found this criterion, as applied to sections of cultures on the chorio-allantoic membrane of the chick embryo, of great value in classifying certain atypical strains of rickettsial diseases in South Africa.

*Pathology, and pathogenicity for animals.* The pathology of spotted fever is in general similar to that of typhus, the essential lesion being a specific endangiitis. Focal brain lesions, formerly believed to be peculiar to typhus, have been shown by Lillie (93) to develop in spotted fever guinea pigs which do not die in the earlier stages of the disease. Previous failure to observe these lesions is probably due to the fact that both human patients and guinea pigs have died before there was time for the lesions to develop.

Monkeys, rabbits and guinea pigs are susceptible to spotted fever, and the pathological picture in these animals is essentially like that seen in man. Dogs are susceptible to infection with fièvre boutonneuse and are probably important in maintaining the disease in nature. A number of other lower animals are capable of harboring the infection, usually in a clinically inapparent form. These hosts include practically all North American rodents, and in South America the wild dog and the opossum.

The scrotal reaction in guinea pigs reacting to highly virulent strains of

spotted fever is usually of a different type from that seen in typhus. Following a brief period of simple swelling and redness, the scrotum in these strains of spotted fever becomes deep red, often with petechial hemorrhages, and eventually dark purplish-black or even gangrenous. A similar scrotal reaction often occurs in human patients. Scrotal sac exudate is not copious, as in murine typhus, but acute arteritis with thrombosis is the outstanding feature of the lesion. These changes occur in guinea pigs injected subcutaneously as well as in those injected intraperitoneally, while the typhus lesion, which is an acute inflammation of the tunica vaginalis, occurs only after intraperitoneal injection.

In virulent spotted fever, rickettsiae are usually not found, or found only in very small numbers, in smears from the tunica surface, but are seen in large numbers in the endothelium and smooth muscle tissue of arterioles beneath the surface, both in the scrotum and in the testis.

Certain milder strains of spotted fever, notably *fièvre boutonneuse* (94) and certain mild strains originating in the United States (61), give an acute exudate with moderate numbers of cells containing rickettsiae in the scrotal sac of the guinea pig, simulating the typhus reaction. Rickettsiae are present in much smaller numbers than in typhus, however, there being rarely more than twenty-five to thirty organisms per cell, and the organisms appear larger and often more lanceolate in shape. Intranuclear rickettsiae are occasionally recognized in smears from the scrotal sac. They have recently been found in considerable numbers in guinea pigs treated with sulfadiazine and sulfathiazole (95).

Certain strains of spotted fever rickettsiae cause practically 100 per cent mortality in the guinea pig, while other strains are never fatal. It is not known whether the virulence for the guinea pig corresponds accurately to the virulence for man. Mildly virulent strains (for the guinea pig) have been recovered in the western United States, as well as in many other parts of the world.

*Strain variation.* Spotted fever, like typhus, occurs in man in various parts of the world and shows considerable clinical variation. In some instances, the clinical variation depends entirely on the severity of the infection, but certain differences are due to biologically modified strains of the etiological agent.

The diseases now recognized as belonging to the spotted fever group are: 1. Rocky Mountain spotted fever; 2. Eastern spotted fever; 3. *fièvre boutonneuse* (94); 4. São Paulo typhus (96, 97); and 5. South African tick-bite fever (92). These names are given here because they represent localized infections which were studied by different observers. The exact relationship between the strains causing these kinds of spotted fever is not yet entirely clear. It has, however, been established by thorough study that there are no important differences between the agents of Rocky Mountain spotted fever, Eastern spotted fever, and São Paulo typhus. (The last is more appropriately called Brazilian spotted fever.) Unless definite immunological differences are demonstrated, *Dermacentroxiens rickettsi*, the name given to the rickettsia of Rocky Mountain spotted fever should be applied to the etiological agents of all three of these diseases. It should be noted, however, that the species of ticks commonly involved in transmission to man is different in the three diseases: *Dermacentor*

*andersonii* for Rocky Mountain spotted fever, *Dermacentor variabilis* for Eastern spotted fever, and *Amblyomma cajennense* for Brazilian spotted fever.

Fièvre boutonneuse differs clinically from the above three diseases in the presence of a localized primary sore and an inflammatory reaction in the regional lymph nodes. Morphologically, in ticks and tissue cultures, the rickettsia of fièvre boutonneuse is identical with that of Rocky Mountain spotted fever (94). Parker (98) has shown, however, that although guinea pigs recovered from either infection are solidly immune to the other, a vaccine made from formalinized tick tissues, which protects guinea pigs effectively against Rocky Mountain spotted fever, Eastern spotted fever, or Brazilian spotted fever, is completely ineffective against fièvre boutonneuse. The author (94) has confirmed this observation with careful attention to the question of dosage. This finding is of great interest, and the underlying mechanism should be investigated further, since it involves a principle which, so far as I am aware, has not been brought to light before. Since the arthropod vector and intermediate mammalian host are different in the two diseases, the observation is reminiscent of that made by Laidlaw and Duncan in distemper, namely that, using the same strain of virus, vaccine made from formalinized ferret tissues, and effective for immunizing ferrets, was ineffective for the immunization of dogs.

This immunological difference justifies one in regarding fièvre boutonneuse as a somewhat modified strain, and the etiologic agent could, as suggested by Alexander and Mason (92), appropriately be called *Dermacentroxenus rickettsi* (var.) *conori*. The differences between the two organisms appear to be of about the same magnitude as those between *Rickettsia prowazeki* var. *prowazeki* and *R. prowazeki* var. *mooseri*.

The identity of tick-bite fever of South Africa has been the subject of considerable dispute, since Pijper (99) has maintained that it is immunologically unrelated to spotted fever. The disease is of very feeble virulence for guinea pigs, and this fact makes the interpretation of cross-immunity tests difficult, particularly in that the slight non-specific immunity caused by an unrelated infection may upset the delicate balance and give false negative results. In the author's opinion, Mason and Alexander (92) by the careful application of a number of available criteria, including the demonstration of intranuclear localization of the causative organism in chick embryo cultures, have established tick-bite fever as a member of the spotted fever group. These workers suggest the name "*Dermacentroxenus rickettsi pijperi*" for the etiological agent of the disease, but it is not clear on just what basis they would separate this organism definitely from that of fièvre boutonneuse. In general, it seems unwise to create variety names unless differences are found which can be demonstrated with regularity by laboratory methods.

*Immunology.* One attack of spotted fever confers lasting immunity against subsequent attacks, and it is known that the vaccine made by Spencer and Parker against Rocky Mountain spotted fever protects also against Eastern spotted fever. Although it is ineffective against fièvre boutonneuse, it is probable that a strictly homologous vaccine made by the same method would be

successful. It would be of interest to find out whether vaccine made from *Dermacentor andersonii* ticks with fièvre boutonneuse rickettsiae would be sufficient, or whether it would be necessary to use the common tick vector of fièvre boutonneuse (*Rhipicephalus sanguineus*).

Several of the methods detailed above for obtaining high concentrations of *R. prowazeki* for vaccine purposes are applicable to spotted fever. Vaccine made from the tissues of ticks by the method of Spencer and Parker (100) is the only one used in practice at present, although Cox (73) believes that the yolk sac method is superior. It should be noted that the tick vaccine for spotted fever antedated Weigl's louse vaccine for typhus (70). Immunity produced by killed rickettsiae of any source is relative rather than absolute, and vaccination should be repeated yearly. Available evidence suggests that the value of vaccination lies partly in the reduction of severity of the infection, which is occasionally, at least, acquired in spite of vaccination.

FitzPatrick (101) has obtained high concentrations of spotted fever rickettsiae by a method similar to that of Castaneda (74).

Passive immunization in spotted fever is experimentally possible, and Topping (102) has produced a hyper-immune rabbit serum for limited clinical use. This serum has not been fully evaluated, but its value on the basis of present data appears somewhat doubtful in severe cases.

It is obvious that unexploited possibilities exist in the way of immunization with living rickettsiae from the milder strains. Tick-bite fever of South Africa, for example, is a disease with no mortality; and its rickettsiae, which have been shown to immunize guinea pigs against fièvre boutonneuse, conceivably might immunize against more virulent strains of spotted fever. Cox (73) has also reported the development of an avirulent strain of spotted fever rickettsiae by prolonged cultivation in the yolk sac. This strain causes in guinea pigs no febrile reaction or other evidence of illness, but produces solid immunity to massive doses of highly virulent rickettsial strains.

**Agglutination reactions.** The Weil-Felix reaction is usually positive in spotted fever, the characteristic finding being an increase from a low or negative reaction to a positive reaction in moderate titre with *Proteus vulgaris* OX 19 and OX K. Positive reactions at a dilution of 1:160 are occasionally seen in other diseases. Specific agglutination with rickettsial suspensions is also possible, the suspensions being made by concentrating the organisms from the yolk sac or other sources. This specific agglutination test has advantages over the Weil-Felix reaction in that it is more reliable and gives a presumptive diagnosis earlier in the course of the disease.

**Latent infection.** The infective agent of spotted fever differs from that of typhus in the fact that it disappears much more rapidly from the tissues of recovered or latently infected experimental animals (80).

**Chemotherapy.** As in typhus, Topping (83) found prontosil and sulfapyridine of no value and possibly even detrimental when administered to guinea pigs with spotted fever. The author with von Hofgaarden (103) has tried sulfathiazole, neoprontosil, sulfadiazine, fuadin, and atabrine in spotted fever guinea

pigs, and obtained negative results, similar in all instances to those reported by Topping but with even greater emphasis on the detrimental effects. In guinea pigs treated with sulfadiazine, intranuclear rickettsiae were for the first time seen in considerable numbers in smears from the scrotal sac and in sections from the testis.

*Diagnosis.* Methods for the diagnosis of atypical strains of spotted fever have been described above in the discussion of strain variation and in the section on typhus fever.

#### RICKETTSIA TSUTSUGAMUCHI (TSUTSUGAMUCHI DISEASE)

*Etiological studies.* Although long suspected of being a rickettsial disease, proof of the etiological relationship of a rickettsia (*R. tsutsugamuchi*) (*R. nipponica*) (*R. orientalis*) to the disease was obtained much later than in the case of typhus and spotted fever. One reason for this is the low susceptibility of experimental animals. In 1928, Ishiware and Ogata (104) transmitted the disease to the rabbit by intratesticular inoculation and demonstrated the rickettsiae in the interstitial cells of the testis. Nagayo and his co-workers (105) showed that intraocular injection in the rabbit resulted in an ophthalmitis, and demonstrated rickettsiae very clearly in the endothelial cells overlying Descemet's membrane.

Proof of the etiological relationship of the organism to the disease is based on its constant demonstration in cells of the above types in rabbits after injection with material from human patients, and also on the cultivation of the organism in tissue culture for long periods of time (106) with full retention of its virulence when introduced into rabbits by the above methods. Although obviously transmitted by the larval stage of mites (*Trombicula akamushi* and *T. deliensis*), no studies of the location and morphology of the organism in mites have been published.

The disease has been less completely studied than typhus and spotted fever, cytological studies being particularly incomplete. For this reason, its exact relationship to the latter two diseases was for a time obscure. Lewthwaite and Savor (107) have, however, demonstrated rather conclusively that tsutsugamuchi rickettsiae are immunologically distinct from those of typhus and spotted fever. An important factor in their work was the fortunate chance of transmitting the disease to guinea pigs with resistance lowered as a result of a vitamin-deficient diet (108), thus gaining the opportunity of carrying out cross-immunity tests with the other rickettsial diseases under approximately similar conditions.

*Size, morphology, and staining.* *R. tsutsugamuchi* shows considerable morphological similarity to certain forms of *R. prowazeki* and *D. rickettsi*. The organism is a short diplobacillus with bipolar staining. Lewthwaite and Savor (108) give the average limits of size as 0.8 to 2.0  $\mu$  in length and 0.3 to 0.5  $\mu$  in width. The organism tends to be shorter and plumper than typhus and spotted fever rickettsiae, and it is believed that differential diagnosis could be made on morpho-

logical grounds alone by an experienced observer. Ogata and Unno (109) depict short chains. The staining reactions are like those of the other rickettsiae, but bipolar staining is perhaps more prominent. The organisms have not been described as capable of distending cells, and are usually diffusely scattered rather than clustered.

*Cultivation, and growth requirements.* Observations in experimental animals and in tissue culture (106) suggest that *R. tsutsugamuchi* is an obligate intracellular parasite, and that its growth requirements are essentially like those of the rickettsiae of typhus and spotted fever. The organism grows freely in the cytoplasm of the interstitial cells of the rabbit testis and the endothelial cells overlying Descemet's membrane. No convincing evidence of intranuclear localization has been presented.

*Pathology, and pathogenicity for animals.* Pathological studies in man have not been reported. From the fact that the cutaneous rash resembles that of typhus and spotted fever, one might conclude that the pathology is probably an endangitis, as in the latter diseases. Lewthwaite and Savor (108) described focal brain lesions in guinea pigs similar to those seen in typhus and spotted fever. The occurrence of ascites in infected guinea pigs, with rickettsiae in cells of the ascitic fluid, was constant in the disease described by these workers. Susceptible animals, in addition to man, are monkeys, rabbits, rats, and guinea pigs, but the disease is very difficult to establish and maintain in these animals (excepting the rabbit following injection by the routes mentioned above.) The vole is apparently the reservoir of the disease in Japan, while in Formosa the rat is the reservoir. Although Lewthwaite and Savor (108) established and maintained a strain in scorbutic guinea pigs, they were unable to repeat this achievement. Progress would be more rapid if an animal highly susceptible to the disease by the ordinary routes of inoculation could be found.

*Strain variation.* Clinically the disease occurs in a mild and a severe form. The severe form is characterized by a necrotic local lesion at the site of attachment of the vector and inflammation of the regional lymph nodes, while these lesions are absent in the mild form. It is of interest to note that this situation is the reverse of that obtaining in the spotted fever group, where the mild forms (fièvre boutonneuse and tick-bite fever) are the ones associated with a severe local reaction. No criteria for the recognition of true variant strains of *R. tsutsugamuchi* have been described. It is clear that tropical typhus of the rural type (rural typhus) is essentially identical with tsutsugamuchi disease.

*Immunology.* The immunity conferred by an attack of tsutsugamuchi disease is apparently less complete than in typhus and spotted fever. Reinfection is fairly common, but second attacks are usually mild. Vaccines could undoubtedly be prepared by the methods detailed above, since the organism grows in tissue culture, and would probably grow in the yolk sac and in other media containing living cells.

The Weil-Felix reaction, which is important in diagnosis, is characterized by agglutination of *Proteus vulgaris* OX K in high serum titre.

## RICKETTSIA RUMINANTIIUM (HEARTWATER)

*Etiological studies.* Heartwater, a highly fatal and economically important disease of cattle, sheep, and goats, was etiologically obscure until Cowdry (110) demonstrated in 1926 the causative agent, *R. ruminantium*, in the endothelial cells of the kidney and brain of sheep dying of the disease. By studies in the tick, *Amblyomma hebraeum*, Cowdry (111) soon demonstrated the inseparability of the organism from infectivity, and established the etiological relationship of the organism to the disease by methods similar to those applied in typhus and spotted fever. The infection is not hereditary in the tick, but is transmitted from the nymph to the larva and from the larva to the adult.

*Size, morphology and staining.* Morphologically, *R. ruminantium* is rather strikingly different from typhus and spotted fever rickettsiae. Rod-shaped forms are infrequent, and most of the organisms are rounded or elliptical, with occasional horseshoe shapes resembling certain forms assumed by *Bartonella canis*. The organisms are confined to the cytoplasm, and occur there in large numbers. They are at times rather diffusely arranged, but often in definite clusters like those of the elementary bodies of psittacosis. The range in size is approximately that of *D. rickettsi*. The organism is not filterable.

On morphological and other grounds, it would perhaps be preferable, on the basis of present knowledge, to assign this organism to a different genus in the family RICKETTSIACEAE, but to do so would serve no useful purpose at the present time.

*Cultivation, and growth requirements.* No reports are available on the cultivation *in vitro* of *R. ruminantium* by any of the methods involving the use of living or surviving cells; and the organism refuses to grow in cell-free media. In ticks and in mammalian tissues, it behaves as an obligate intracellular parasite. Virulence of infected tissues at room temperature is retained for only a few hours.

*Pathology, and pathogenicity for animals.* Pathologically, *R. ruminantium* appears to resemble the rickettsiae of typhus and spotted fever in that it infects primarily the vascular endothelium. A striking difference from the above diseases is the accumulation of large quantities of fluid in the pleura, peritoneum, and pericardium (whence the name heartwater). The liver, spleen, and kidneys are congested. Microscopically, there are slight perivascular accumulations of inflammatory cells, but no thromboses. The rickettsiae are seen in sections in the endothelium of small blood vessels, particularly in the brain and kidneys, and can be most readily demonstrated in smears made by scraping the endothelium of large blood vessels.

Small laboratory animals are said not to be susceptible (Alexander, Mason, and Neitz (92), although Balozet (112) claims to have carried the infection for several generations in guinea pigs, rabbits, and rats.

*Immunology.* In recovered animals, even after 105 days, rickettsiae are still demonstrable in the vascular endothelium (113) and the disease may be transmitted from sheep to sheep by scrapings of the endothelium at a time when the blood is no longer infective. The immunity appears to be that of tolerance;

and since immunity is only gained by active infection, no satisfactory method of vaccination has been devised. Immune serum treatment has likewise been found of no avail, and inoculation with attenuated virus has given no results of practical value. It is said that immunity may be broken down by inoculation with a different strain of the disease (113).

#### RICKETTSIA WOLHYNICA (TRENCH FEVER)

*Etiological studies.* The role of the body louse in transmitting trench fever in man was established during the world war of 1914–1918 by British and American Commissions (114, 115). The disease was readily transmitted to human volunteers by rubbing the feces of infected lice into the scarified skin and also by allowing infected lice to feed on the volunteers. In 1916, Töpfer (8, 9) described extracellular rickettsiae in the intestines of lice, and suggested that these organisms might be the cause of the disease. Similar extracellular organisms were described by Munk and da Rocha-Lima (10) in presumably normal lice and named *Rickettsia pediculi*.

Arkwright, Bacot, and Duncan (116) showed that lice proven non-infective and free from rickettsiae regularly became infective about nine days after feeding on patients with trench fever, and that extracellular rickettsiae regularly appeared in the intestinal tract and feces of such infective lice. While Bacot was studying typhus fever in Poland, he acquired trench fever (7). Stock lice, entirely free from microorganisms of any sort, were being fed on Bacot at the time. Twenty days after the onset of his illness, extracellular rickettsiae appeared in these lice and also in other lots of stock lice fed on him at the time.

Evidence of the etiological relationship of the organism *Rickettsia wolhynica* (also called *R. quintana*) to trench fever is thus of an incomplete nature, and it can only be said that the organism is probably the cause of trench fever. The failure to transmit the infection to experimental animals and the practically complete disappearance of the disease following the war of 1914–1918 have made further progress impossible. Recently, the disease has been reported (117) in individuals on whom presumably normal lice were being fed in the process of making typhus vaccine by the Weigl method.

*Morphology and staining.* *R. wolhynica* is somewhat more deeply stained in film preparations than the more characteristic rickettsiae. The organisms are also somewhat plumper, more definitely oval, and less pleomorphic than typhus rickettsiae. It would, however, be difficult to distinguish *R. wolhynica* from *R. prowazeki* on morphological grounds alone.

*Growth requirements.* *R. wolhynica* has not been cultivated in cell-free media. It is more resistant to heat and drying than *R. prowazeki*, resisting dry heat at 80 C for twenty minutes and desiccation in sunlight for four months. In the intestinal tract of lice, the organism is concentrated along the borders of the lining cells, suggesting that it may depend for its growth on some product of the metabolism of these cells. Modern methods of cultivation in media containing living cells, such as those detailed above, have never been applied to

the study of *R. wolhynica*, and it seems probable that much information would be gained by the use of these methods.

*Immunology.* The disease is characterized by relapses which may occur as late as two years after onset. Immunity is slow in development and is probably the immunity of tolerance, since recovered cases continue to infect lice, previously free from organisms, for many months.

#### RICKETTSIA BURNETI (DIAPORICA). (Q-FEVER)

*Etiological studies.* In 1937, Burnet and Freeman (118), in studying an epidemic among individuals working in slaughter houses or on dairy farms in Australia, were able to transmit the disease to experimental animals, and they described a rickettsia-like organism in smears from the spleens of infected mice. This organism was named *Rickettsia burneti* by Derrick (119). A similar organism was recovered by Davis and Cox (120) from ticks (*Dermacentor andersonii*) collected in Montana. This organism was found to be pathogenic for guinea pigs, and its properties were carefully studied before there was any indication that it might be related to human illness. It was called *Rickettsia diaporica*. Dyer (121) reported a case of accidental laboratory infection with *R. diaporica*, and showed that there was cross-immunity between Australian Q-fever and the Montana infection in guinea pigs (122). This observation has been confirmed and careful comparison of the two organisms, *R. burneti* and *R. diaporica*, has shown that they are closely related if not identical. Minor differences in behavior in guinea pigs, not unlike the differences described in various strains of spotted fever, have been observed, but no differences inconsistent with the fundamental similarity of the two organisms. The etiological relationship of *R. burneti* and *R. diaporica* to Q-fever in Australia and in the United States, respectively, has been established by guinea pig inoculation and serological tests. There have been several cases of laboratory infection, both in Australia and in the United States, and recently an institutional outbreak occurred in Washington, D. C. (123).

*Filterability, morphology, and staining.* Workers in both countries have studied the biological properties of the organisms and found them to be filterable through Berkefeld filter which apparently did not allow typhus and spotted fever rickettsiae to pass (120). This observation is the basis for the specific name "diaporica" given by the American investigators. Uncertainty exists regarding the occurrence of the organisms in an invisible form.

Morphologically the rickettsiae of Q-fever are similar to those of spotted fever and typhus, but appear somewhat larger (perhaps because they stain more deeply). The organisms, in infected animal tissues, apparently grow both intracellularly and extracellularly. Within infected cells, which are chiefly mesothelial and reticulo-endothelial cells, the organisms tend to form compact spherical clusters, the pattern of infection being quite similar to that of *Bartonella bacilliformis*.

*Cultivation, and growth requirements.* Growth occurs, both intracellularly and extracellularly, in the various media containing living cells commonly used for

the growth of rickettsiae and filterable viruses (124). Multiplication has not occurred in any cell-free medium. Particularly massive growth is obtained in the yolk sac of the developing chick embryo. The culture requirements of *R. diaporica* appear to be similar to those of *Bartonella bacilliformis*, but no multiplication occurs in cell-free media which have been successfully used for the latter organism. The same can be said for *Bartonella muris*, however, which closely resembles *B. bacilliformis*, and it seems probable that *R. diaporica* will eventually be cultivated in a cell-free medium. Careful cytological studies in tissue cultures have not been carried out.

*Pathology, and Pathogenicity for animals.* In a fatal human case reported by Lillie, Perrin, and Armstrong (125), the gross pathological findings were pulmonary edema and congestion, firm granular consolidation of the upper lobe of the right lung posteriorly, and a large soft spleen. The other organs showed no important changes. Microscopically, the picture in the lung was that of an atypical pneumonia, with much fibrin in the alveoli and bronchioles, and in general a scanty mononuclear cell reaction instead of the purulent reaction seen in typical bacterial pneumonia. Similar pathological changes were seen in the lungs of experimentally infected monkeys. In neither the human nor the monkey lungs were rickettsiae demonstrated histologically. The similarity of the pathologic picture to that described in fatal cases of atypical pneumonia of unknown origin by Longcope (126) and by Kneeland and Smetana (127) was striking. The picture is also rather similar to that of certain cases of psittacosis pneumonia (128).

In guinea pigs, after an incubation period of two to eighteen days depending on the amount of infective material injected, fever follows for four to six days, and loss of weight and appetite. Guinea pigs have been successfully infected by the inoculation of human blood or urine. There is practically no mortality in the guinea pig, but at necropsy a large soft spleen is found and rickettsiae may be seen, both free and in mononuclear cells, in smears of the spleen stained by the Giemsa or the Machiavello method.

In the guinea pig, Lillie (129) has described perivascular accumulations of lymphocytes and rare focal brain lesions like those seen in typhus and spotted fever. Granulomatous tubercle-like lesions, with giant cells, are also described in the spleen, liver, and other organs.

In mice, Burnet and Freeman (118) have described small, necrotic foci in the liver, and the presence of rickettsiae in the Kupffer cells of that organ.

*Immunology.* Vaccines prepared from yolk sac suspensions or from infected mouse spleens (130) are effective in building up active immunity. Hyperimmune serum also gives considerable protection. In general, the immunological aspects of the disease appear to be similar to those in typhus and spotted fever.

#### RICKETTSIA CANIS AND RELATED ORGANISMS

*Rickettsia ovina* was observed in sheep in 1930 by Lestoquard and Donatien (131). Later, a similar organism (*R. canis*) was reported in dogs (132),

and a third variety (*R. bovis*) in cattle (133). These three organisms are morphologically identical. They all produce febrile illness in their mammalian hosts and have in common the fact that they parasitize the monocytes of the circulating blood. Evidence suggesting tick transmission has been presented in the case of all three organisms, but morphological studies of the organisms in their probable vectors have not been reported.

*Rickettsia ovina* was first found in monocytes of the circulating blood from a sheep suffering from a febrile illness after inoculation with emulsions of organs of the tick, *Rhipicephalus bursa*. A second sheep in which the rickettsiae were seen had a concomitant infection with *Babesiella ovis*. A third instance of infection with *R. ovina* was encountered in a splenectomized sheep inoculated with blood from a second splenectomized sheep in which a double infection with *Babesiella ovis* and *Anaplasma ovis* was present. *Rickettsia ovina* has been very incompletely studied, and it is impossible to draw any conclusions regarding its exact nature.

*Rickettsia bovis*, similarly, was found in the circulating monocytes of a bull on which ticks of the genus *Hyalomma* had been fed. The ticks were infected with *Theileria dispar* and had transmitted this organism to the bull. The infection with *R. bovis* was presumably derived from the ticks.

*Rickettsia canis* has been studied in somewhat greater detail. It was first found in the circulating blood of dogs suffering from a fatal illness which began a few days after many ticks (*Rhipicephalus sanguineus*) had fed upon them. Later the infection was found to be common in dogs and to occur in both an acute and a chronic form. Ticks fed on dogs suffering from the infection were shown to be infectious when ground up and injected into other dogs. In one instance, infection was produced in a monkey by the inoculation of ground-up larvae issuing from a female *Rhipicephalus sanguineus* which had engorged on a dog suffering from acute infection with *Rickettsia canis*.

Morphologically, *R. canis*, like the other two members of the group "rickettsiae of monocytes," occurs usually in compact intracytoplasmic aggregations or groups, often indenting the nucleus but never invading it. Several clusters may occur in a cell, the pattern produced simulating that of *Bartonella bacilliformis*, *Rickettsia diaporica* and the organism of psittacosis. The individual elements are 0.2 to 0.3  $\mu$  in diameter when closely packed, but range up to 0.5 or 0.6  $\mu$  in diameter when separated from one another. They are rounded, elliptical, polygonal or rarely coccobacillary or bacillary. The staining reactions are similar to those of the typical rickettsiae, except that the structures do not stain by Castaneda's method. Filtration experiments gave negative results.

The infection is transmissible to dogs and monkeys by intravenous or subcutaneous injection of infected blood or emulsions of lung, spleen, or brain tissue. Dogs which recover from the infection remain chronically and latently infected, and show an immunity of tolerance, similar to that seen in infection with *R. ruminantium*. Relapses can be brought about by intercurrent infection or by splenectomy.

*R. canis* appears to be distinct from bartonella, eperythrozoon, hepatozoon,

and piroplasma, and is obviously unrelated to the rickettsia of fièvre boutonneuse. In view of the fact that *R. canis*, *R. ovina* and *R. bovis* are primarily parasites of the circulating monocytes, it is the author's opinion that more detailed studies must be carried out before a decision can be reached as to whether these organisms should be grouped with the rickettsiae. If they should be so classed, they should be given generic names other than Rickettsia. These organisms have been discussed here largely for the sake of completeness. A detailed account of them may be found in a paper by Donatien and Lestoquard (113).

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